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A SHOWDOMYCIN-RESISTANT MUTANT OF *ESCHERICHIA COLI* K-12 WITH ALTERED NUCLEOSIDE TRANSPORT CHARACTER

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SUMMARY

1. Two deoxycytidine transport systems were found in *Escherichia coli* K-12: one has a high affinity for deoxycytidine and is not significantly inhibited by guanine and hypoxanthine nucleosides, the other has a low affinity for deoxycytidine and is markedly inhibited by these nucleosides.

In a showdomycin-resistant mutant (Shm^r-001), the former system was no longer found to be present while the latter was found to remain active.

2. Of the two deoxyguanosine transport systems in *Escherichia coli* K-12, one was found to be markedly inhibited by deoxycytidine whereas the other was not affected by the nucleoside.

In the mutant (Shm^r-001), the former system was lost but the latter remained active.

3. In the case of adenosine transport in *Escherichia coli* K-12, one system had a high affinity for adenosine and was inhibited by deoxyadenosine, deoxycytidine and deoxythymidine, while the other system had low affinity for adenosine and was only slightly inhibited by these nucleosides. The former system was lost in the mutant (Shm^r-001) but the latter remained active. Both systems were inhibited by azide and *p*-chloromercuribenzoic acid.

INTRODUCTION

A wide variety of nucleoside transport systems have been suggested for *Escherichia coli*[1,2]. Although a number of methods have been employed to show the multiplicity of nucleoside transport systems, a clearer proof of this multiplicity may be expected from studies on a mutant lacking one of these transport systems.

We have isolated showdomycin-resistant mutants from *Escherichia coli* K-12[3]. These mutants have an impaired ability to take up not only showdomycin but also a number of nucleosides including adenosine, deoxyadenosine, deoxyguanosine, uridine, cytidine, deoxycytidine and deoxythymidine. No significant amount of showdomycin was taken up by these mutants, and all ordinary nucleosides were taken up at a very slow rate. This suggested that there may be at least two transport systems for each nucleoside, one which is common for a wide variety of nucleosides and one which is more specific for the respective nucleosides.

In this paper we describe a comparison between a showdomycin-resistant mutant (Shm^r-001) and the parent (K-12) cells with regard to the ability to metabolize nucleosides, the effect of other nucleosides on the entry of a particular nucleoside, the kinetics of the entry of adenosine, and the effect of cold-osmotic shock on the ability to take up deoxycytidine and showdomycin.

MATERIALS AND METHODS

Organisms and media

The showdomycin-resistant mutant, Shm^r-001, was isolated from *Escherichia coli* K-12 as previously reported[3] and used throughout this study. This mutant can grow even in the presence of 400 μM of showdomycin (Fig. 1). A synthetic minimal medium as described by Davis and Mingioli[4] was used throughout this study.

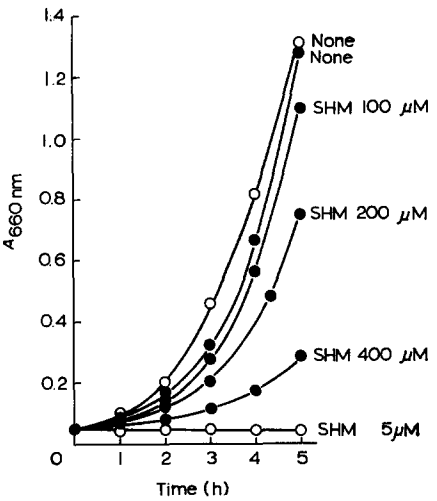

Fig. 1. Effect of showdomycin on cell growth of the parent (K-12) and mutant (Shm^r-001) strains. Exponentially grown cells of the parent (K-12) or mutant (Shm^r-001) were suspended in minimal medium to give a final concentration of $0.8 \cdot 10^8$ cells per ml, and incubated with the cited amounts of showdomycin. At predetermined intervals, the bacterial cell growth was followed turbidimetrically at 660 nm. ○—○, parent; ●—●, mutant. SHM, showdomycin.

Measurement of the uptake of [^{14}C]showdomycin and other labeled compounds

This was carried out by rapid chilling and rapid Millipore filtration techniques as previously described[5].

Measurement of deamination of deoxycytidine and adenosine by Escherichia coli cells

Cell suspensions of parent and mutant strains were incubated with [^{14}C]deoxycytidine or [^{3}H]adenosine in minimal medium at 25 °C or 37 °C. After a given time, the incubation mixtures were filtered through a Millipore filter (Type HA, 0.45 μm pore size). The filtrates were mixed with the appropriate non-labeled reference nucleosides and bases, and spotted on Whatman 3MM paper strips (2.5 cm × 40 cm). The paper strips were developed for 4 h at 22 °C by ascending chromatography with Solvent I (ethylacetate–water–formic acid, 60:35:5, v/v/v), Solvent II (ethylacetate–

1-propanol–water, 4:1:2, v/v/v) or Solvent III (water adjusted to pH 10 with 1 M NH_4OH) as stated in the legends to the figures. To identify the radioactive products on the paper chromatograms more conveniently, the spots of non-labeled reference compounds were located under an ultraviolet lamp, after which the chromatograms were cut into pieces (0.5 cm) and counted in a toluene phosphor solution by a liquid scintillation spectrometer.

Osmotic shock treatment

The procedure described by Nossal and Heppel[6] was slightly modified. About $5.1 \cdot 10^{10}$–$6.9 \cdot 10^{10}$ cells harvested in the mid-logarithmic phase of growth were suspended in 7 ml of 0.03 M Tris–HCl buffer (pH 7.3). The cell suspension was then mixed with 6 ml of 0.03 M Tris–HCl buffer (pH 7.3) containing 40 % sucrose and 0.2 mM EDTA. The mixture was shaken for 10 min at 22 °C and centrifuged at 14500 × *g* for 12 min at 10 °C. The pellet was rapidly dispersed in 12 ml of ice-cold 0.5 mM $MgCl_2$ (aqueous solution) and swirled by a glass stick for 15 min in an ice-bath. The mixture was centrifuged at 14500 × *g* for 12 min. The supernatant obtained is called "shock fluid". The pellet was resuspended in ice-cold 0.01 M Tris–HCl buffer (pH 7.3) containing 0.15 M NaCl and 0.5 mM $MgCl_2$ at a final concentration of $3.2 \cdot 10^8$–$6.9 \cdot 10^8$ cells per ml; the cell suspension is called "shocked cell" suspension.

Reconstitution of transport system

The parent and mutant shocked cell suspensions were incubated with an equal volume of the shock fluid of the parent or mutant cells for 6 min at 37 °C, and, after storage in an ice-bath, the abilities to take up [^{14}C]showdomycin, [^{14}C]deoxycytidine, [^{14}C]uracil, and [^{14}C]leucine were determined under standard conditions.

Chemicals and reagents

[^{14}C]Showdomycin was obtained by incubating *Streptomyces showdoensis* with sodium [2-^{14}C]acetate[5,7]. The labeled compounds were from Schwarz BioResearch Corp.; the unlabeled nucleosides and bases from Sigma Chemical Corp. and Schwarz BioResearch Corp.

RESULTS

Reduced ability of showdomycin-resistant mutant cells to metabolize deoxycytidine and adenosine

Fig. 2 shows the paper chromatograms of culture filtrates of the parent and mutant cells obtained after incubation with [^{14}C]deoxycytidine or [^{3}H]adenosine for 6 min at 25 °C. It can be seen that in the culture filtrate of parent cells the greater part of added deoxycytidine and adenosine was found to be changed to uracil and inosine, respectively, whereas in the culture filtrate of the mutant cells the greater part of added deoxycytidine and half the amount of added adenosine remained intact under identical conditions. If it is assumed that cytidine deaminase (EC 3.5.4.5), deoxythymidine phosphorylase (EC2.4.2.4) and adenosine deaminase (EC 3.5.4.4) are located inside the cell membranes, then these results may indicate that the rates of entry of deoxycytidine and adenosine into the mutant cells are much slower than those into parent cells at low concentrations of these nucleosides.

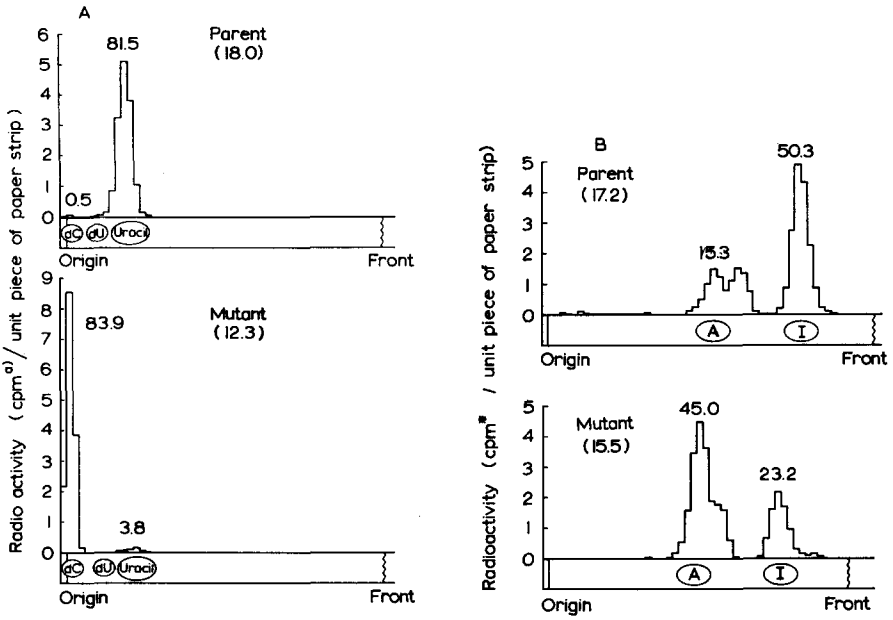

Fig. 2. Paper chromatograms of the culture filtrates when incubated with [^{14}C]deoxycytidine or [^{3}H]adenosine. (A) 1 ml of the cell suspension of the parent ($7.2 \cdot 10^8$ cells/ml) or mutant ($7.0 \cdot 10^8$ cells/ml) strain was incubated with [2-^{14}C]deoxycytidine ($2481 \cdot 10^3$ cpm/μmole, 8.4 μM final concentration) at 25 °C for 6 min. The culture broth was immediately filtered through a Millipore filter. The filtrates (each 120 μl) were applied on strips of Whatman 3MM paper and chromatographed with Solvent I at 22 °C for 4 h by the ascending method. (B) 1 ml of the cell suspension of the parent ($7.2 \cdot 10^8$ cells/ml) or mutant ($7.0 \cdot 10^8$ cells/ml) strain was incubated with [8-^{3}H]-adenosine ($2401 \cdot 10^4$ cpm/μmole, 8.2 μM final concentration) at 25 °C for 6 min. The culture filtrates (each 120 μl) were applied on strips of Whatman 3MM paper and chromatographed with Solvent III at 22 °C for 4 h by the ascending method. The numbers shown in the figures represent the amounts of radioactivity located in each fraction in % of total counts. The numbers in parentheses represent the amount of radioactivity retained in the cells (in % of total counts). dC, deoxycytidine; dU, deoxyuridine. * $\times$ 10^{-2}.

Effect of other nucleosides on [^{14}C]*deoxycytidine and* [^{14}C]*deoxyguanosine uptake*

Fig. 3 shows the effects of other nucleosides on the uptake of [^{14}C]deoxycytidine by the parent and mutant strains. It is worth noting that in the parent strain [^{14}C]-deoxycytidine uptake was inhibited by adenine and pyrimidine nucleosides (adenosine, deoxyadenosine, uridine, deoxyuridine, cytidine, deoxythymidine, 5-iodouridine and 5-bromodeoxyuridine) but not by guanine and hypoxanthine nucleosides (guanosine, deoxyguanosine and inosine). In the mutant strain, however, [^{14}C]deoxycytidine uptake was markedly inhibited not only by the adenine and pyrimidine nucleosides but also by the guanine and hypoxanthine nucleosides. It is also notable that xanthosine did not inhibit the uptake of [^{14}C]deoxycytidine by either the parent or mutant strains.

Table I shows the effects of adenosine and guanosine on the amount of [^{14}C]-deoxycytidine entering into parent and mutant cells. If it is assumed that the sum of the amount of [^{14}C]deoxycytidine changed to [^{14}C]uracil and the amount of [^{14}C]-deoxycytidine retained in the cells represents the amount of [^{14}C]deoxycytidine which entered the cells, then the data in the table suggest that [^{14}C]deoxycytidine

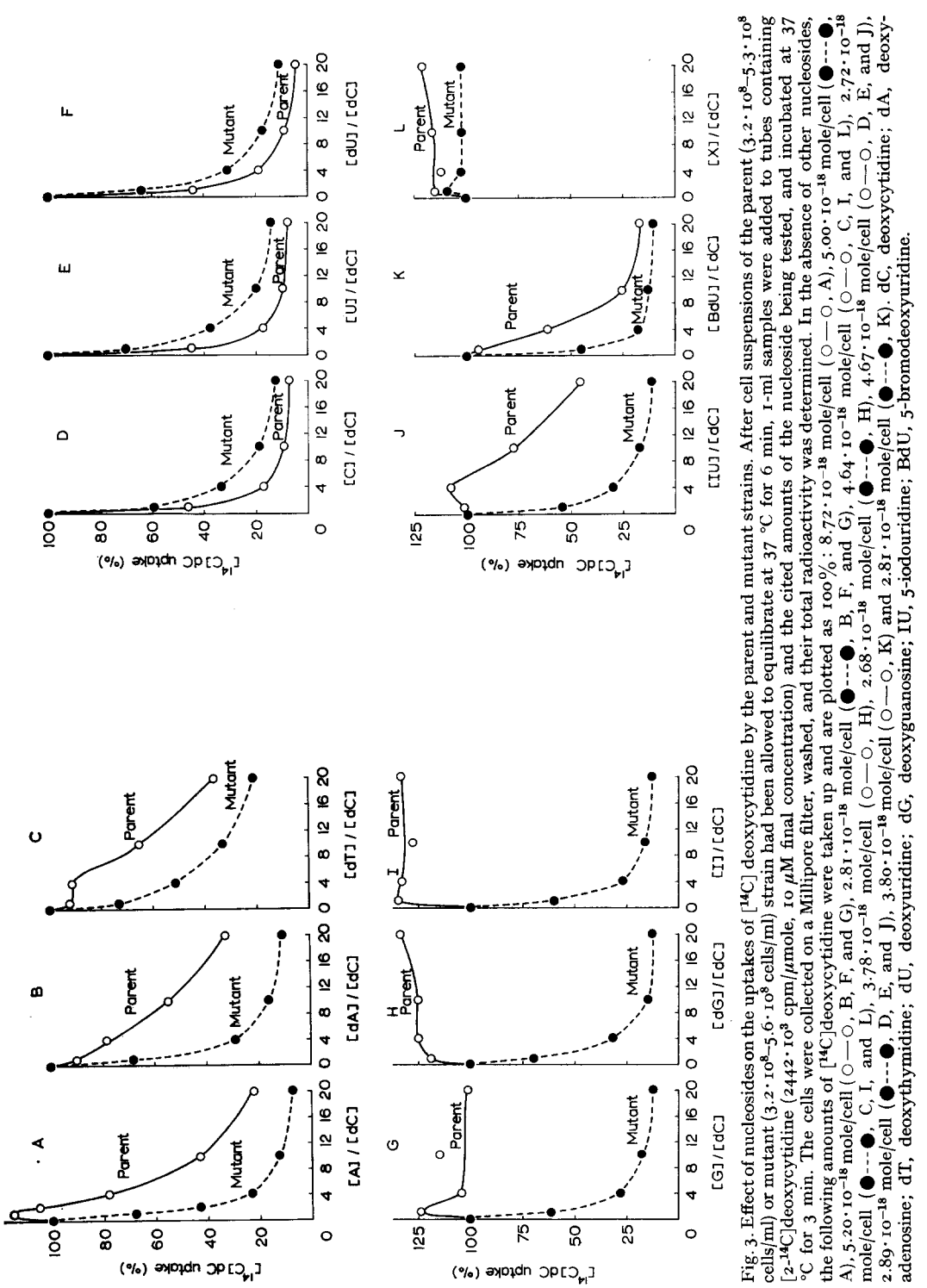

Fig. 3. Effect of nucleosides on the uptakes of [^{14}C] deoxycytidine by the parent and mutant strains. After cell suspensions of the parent ($3.2 \cdot 10^8$–$5.3 \cdot 10^8$ cells/ml) or mutant ($3.2 \cdot 10^8$–$5.6 \cdot 10^8$ cells/ml) strain had been allowed to equilibrate at 37 °C for 6 min, 1-ml samples were added to tubes containing [2-^{14}C]deoxycytidine ($2442 \cdot 10^3$ cpm/μmole, 10 μM final concentration) and the cited amounts of the nucleoside being tested, and incubated at 37 °C for 3 min. The cells were collected on a Millipore filter, washed, and their total radioactivity was determined. In the absence of other nucleosides, the following amounts of [^{14}C]deoxycytidine were taken up and are plotted as 100%: $8.72 \cdot 10^{-18}$ mole/cell (○—○, A), $5.00 \cdot 10^{-18}$ mole/cell (●---●, A), $5.20 \cdot 10^{-18}$ mole/cell (○—○, B, F, and G), $2.81 \cdot 10^{-18}$ mole/cell (●---●, B, F, and G), $4.64 \cdot 10^{-18}$ mole/cell (○—○, C, I, and L), $2.72 \cdot 10^{-18}$ mole/cell (●---●, C, I, and L), $3.78 \cdot 10^{-18}$ mole/cell (○—○, H), $2.68 \cdot 10^{-18}$ mole/cell (●---●, H), $4.67 \cdot 10^{-18}$ mole/cell (○—○, D, E, and J), $2.89 \cdot 10^{-18}$ mole/cell (●---●, D, E, and J), $3.80 \cdot 10^{-18}$ mole/cell (○—○, K) and $2.81 \cdot 10^{-18}$ mole/cell (●---●, K). dC, deoxycytidine; dA, deoxyadenosine; dT, deoxythymidine; dU, deoxyuridine; dG, deoxyguanosine; IU, 5-iodouridine; BdU, 5-bromodeoxyuridine.

entry into the parent cells is markedly reduced by adenosine but only slightly by guanosine at low concentrations, whereas [^{14}C]deoxycytidine entry into the mutant cells is markedly reduced not only by adenosine but also by guanosine under identical conditions.

TABLE I

EFFECT OF ADENOSINE AND GUANOSINE ON THE AMOUNT OF [^{14}C]DEOXYCYTIDINE ENTERING INTO PARENT AND MUTANT CELLS

After cell suspensions of parent ($2.7 \cdot 10^8$ cells/ml) or mutant ($2.6 \cdot 10^8$ cells/ml) strain had been allowed to equilibrate at 37 °C for 6 min, 1-ml samples were added to tubes containing [2-^{14}C]-deoxycytidine ($2481 \cdot 10^3$ cpm/μmole, 9.7 μM final concentration), and incubated with or without adenosine or guanosine (each at a final concentration of 38.1 μM) at 37 °C for 3 min. The culture broths were immediately filtered through Millipore filters. The filtrates (240 μl each) were applied on strips of Whatman 3MM paper and chromatographed with Solvent II at 22 °C for 4 h by the ascending method. For further explanation, see text.

Strain	*Nucleoside added* (μM)	% *of total count*			
		[^{14}C]*Deoxycytidine uptake* (*a*)	[^{14}C]*Uracil formed* (*b*)	$a + b$	[^{14}C]*Deoxycytidine remaining*
Parent	None	9.1	72.7	(81.8)	18.2
	Adenosine (38.1)	9.2	3.3	(12.5)	87.5
	Guanosine (38.1)	12.7	64.6	(77.3)	22.7
Mutant	None	8.4	0.5	(8.9)	91.0
	Adenosine (38.1)	3.0	1.1	(4.1)	96.0
	Guanosine (38.1)	3.6	1.0	(4.6)	95.5

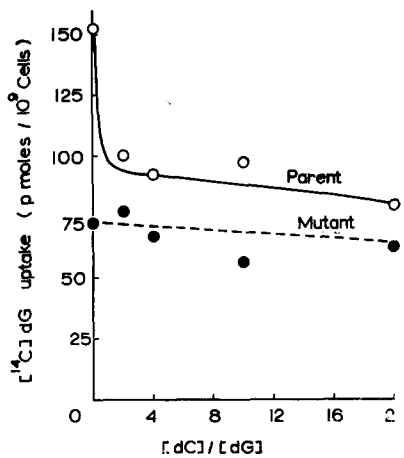

Fig. 4. Effect of deoxycytidine on the uptake of [^{14}C]deoxyguanosine by the parent and mutant strains (at 0.5 °C). After a cell suspension of parent strain ($4.3 \cdot 10^8$ cells/ml) had been allowed to equilibrate for 30 min in an ice-bath, 1-ml samples were added to tubes containing [8-^{14}C]deoxyguanosine ($1467 \cdot 10^4$ cpm/μmole, 11.4 μM final concentration) and the cited amounts of deoxycytidine, and incubated for 10 min in an ice-bath. The cells were immediately collected on a Millipore filter, washed, and their total radioactivity was determined. dC, deoxycytidine; dG, deoxyguanosine.

Fig. 4 shows the effect of deoxycytidine on the uptake of [^{14}C]deoxyguanosine at low temperature (0.5 °C). Since the rate of [^{14}C]deoxyguanosine uptake decreased at low incubation temperature, the difference in uptake of [^{14}C]deoxyguanosine by parent and mutant cells could be examined more accurately at low temperature. The uptake of [^{14}C]deoxyguanosine by the parent strain decreased with increasing deoxycytidine concentration to about one-half of the maximal value, no further decrease being observed. The uptake of [^{14}C]deoxyguanosine by the mutant strain was only half that of the parent strain and was not inhibited by deoxycytidine at all. These results suggest that at least two systems may function for deoxyguanosine transport: one which is inhibited by deoxycytidine and another which is not, the former system being lost in the mutant strain but the latter system remaining active.

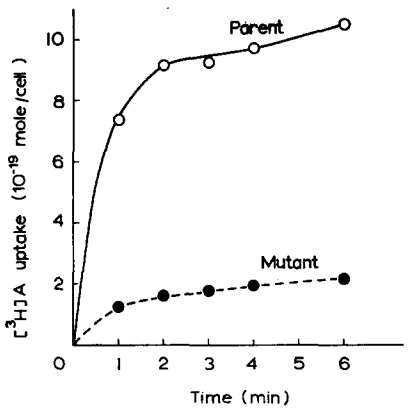

Fig. 5. Time courses of the uptake of [^{3}H]adenosine by the parent and mutant strains in the presence of caffeine. Cell suspensions of the parent ($3.8 \cdot 10^8$ cells/ml) or mutant ($3.5 \cdot 10^8$ cells/ml) strain were incubated with [8-^{3}H]adenosine ($21467 \cdot 10^3$ cpm/μmole, 9.0 μM final concentration) and caffeine (9.8 mM final concentration) at 25 °C. 1-ml aliquots were removed after identical periods and prepared for counting as described in the legend to Fig. 3.

[^{3}H]Adenosine uptake in the presence of caffeine

Peterson *et al.*[2] reported that in caffeine-treated *Escherichia coli* cells, although the entry of adenosine and the deamination of adenosine to inosine proceeded essentially at a normal rate, subsequent metabolism and efflux of inosine were no longer observable. We compared the uptake of [^{3}H]adenosine by parent and mutant cells in the presence of caffeine to obtain information on the adenosine transport without regard to the influence on subsequent metabolism.

Fig. 5 shows typical curves for [^{3}H]adenosine uptake by the parent and mutant strains during incubation with a high concentration of caffeine at 25 °C. [^{3}H]Adenosine was taken up by the parent cells very rapidly in the initial 1–2 min; thereafter the rate of uptake decreased and reached a plateau. The rate and the amount of [^{3}H]-adenosine taken up by the mutant cells were considerably lower than those observed for the parent strain.

Double reciprocal plots of [^{3}H]adenosine uptake in the parent strain were found to be biphasic (Fig. 6A). In the mutant strain, such plots were resolved into a single component for adenosine uptake, as shown in Fig. 6B. These data provide

strong evidence for the existence of (at least) two functional transport systems mediating the entry of exogenous adenosine into parent cells, suggesting that the mutant strain retains only one of these two systems, a low-affinity adenosine transport system (in the presence of caffeine).

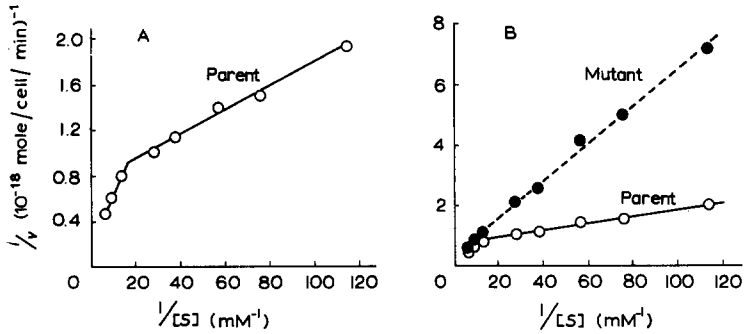

Fig. 6. Double reciprocal plots relating the rate of uptake of [^{3}H]adenosine in the presence of caffeine to concentration in the parent and mutant strains. After cell suspensions of the parent ($2.7 \cdot 10^8$ cells/ml) or mutant ($2.6 \cdot 10^8$ cells/ml) strain had been allowed to equilibrate at 25 °C for 6 min, 1-ml samples were added to tubes containing the cited amounts of [8-^{3}H]adenosine (over a 16-fold range) and caffeine (8.8 mM final concentration), and incubated at 25 °C for 1 min. The cells were immediately filtered, washed, and their total radioactivity was determined.

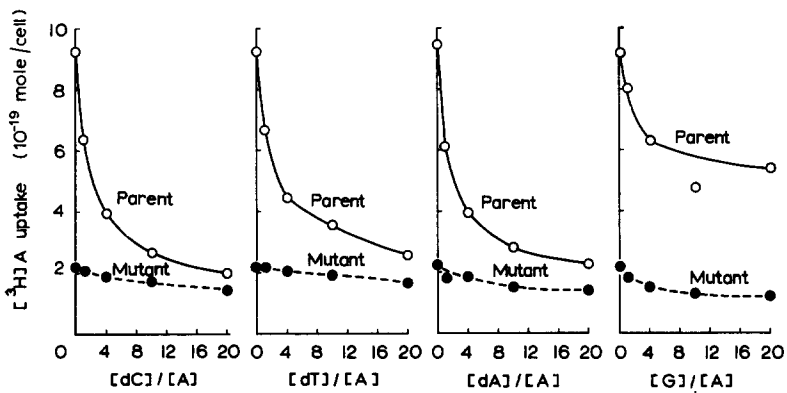

Fig. 7. Effect of nucleosides on the uptake of [^{3}H]adenosine by the parent and mutant strains (in the presence of caffeine). After cell suspensions of the parent ($3.1 \cdot 10^8$–$3.4 \cdot 10^8$ cells/ml) or mutant ($3.2 \cdot 10^8$–$3.3 \cdot 10^8$ cells/ml) strain had been allowed to equilibrate at 25 °C for 6 min, 1-ml samples were added to tubes containing [8-^{3}H]adenosine ($21467 \cdot 10^3$ cpm/μmole, 8.3 μM final concentration), caffeine (8.3 mM final concentration) and the cited amounts of the nucleoside being tested. After a 3-min incubation, cells were filtered, washed, and their total radioactivity was determined. dC, deoxycytidine; dT, deoxythymidine; dA, deoxyadenosine.

Further evidence for the existence of two transport systems was revealed by large differences in susceptibility to other nucleosides (Fig. 7). It was readily observed that in the parent strain much of the [^{3}H]adenosine uptake was inhibited by deoxyadenosine, deoxycytidine and deoxythymidine but the remaining small amount of [^{3}H]adenosine uptake was highly resistant to inhibition by these nucleosides. Guanosine slightly inhibited the uptake of [^{3}H]adenosine by the parent strain. In the mutant strain the amount of [^{3}H]adenosine uptake was smaller than in the

parent strain, and was much more resistant to the inhibitory action of deoxyadenosine, deoxycytidine and deoxythymidine. Thus it seems likely that uptake by the mutant strain corresponds to the smaller portion of the [^{3}H]adenosine uptake by the parent strain which is resistant to the inhibitory action of deoxyadenosine, deoxycytidine and deoxythymidine.

TABLE II

EFFECT OF VARIOUS METABOLIC INHIBITORS ON THE UPTAKE OF [^{3}H]ADENOSINE IN THE PRESENCE OF CAFFEINE BY PARENT AND MUTANT STRAINS

After cell suspensions of parent ($3.3 \cdot 10^8$ cells/ml) or mutant ($3.3 \cdot 10^8$ cells/ml) strain had been allowed to equilibrate at 25 °C for 6 min, 1-ml samples were added to tubes containing [8-^{3}H]-adenosine ($2401 \cdot 10^4$ cpm/μmole, 8.3 μM final concentration), caffeine (8.3 mM final concentration) and the cited amounts of the metabolic inhibitor being tested. After a 3-min incubation, the cells were filtered, washed, and their total radioactivity determined. For further explanation, see text.

Inhibitor (mM)		[^{3}H]*Adenosine uptake* (%)	
		Parent	*Mutant*
None		100 *	100 **
N-Ethylmaleimide	0.08	100.4	74.7
	0.33	124.1	71.7
	1.42	103.5	72.3
p-Chloromercuribenzoic acid	0.017	102.3	82.5
	0.066	43.2	63.9
Iodoacetic acid	0.83	114.5	58.4
	3.30	136.3	45.2
NaN_3	16.6	72.0	71.7
	66.4	35.6	50.0
2,4-Dinitrophenol	0.33	136.8	88.6
	0.85	96.5	88.5
$NaHAsO_4$	82.6	99.3	86.1
	212.7	76.6	60.8

* 100 = $8.42 \cdot 10^{-19}$ mole/cell per 3 min.
** 100 = $1.66 \cdot 10^{-19}$ mole/cell per 3 min.

The effect of various metabolic inhibitors on [^{3}H]adenosine uptake was studied in the parent and mutant strains. As indicated in Table II, a marked difference between the parent and mutant strains was observed for the effect of iodoacetic acid on [^{3}H]adenosine uptake: [^{3}H]adenosine uptake by the parent strain was stimulated by iodoacetic acid at the concentrations examined, whereas at similar iodoacetic acid concentrations the uptake of [^{3}H]adenosine by the mutant strain was markedly inhibited. [^{3}H]Adenosine uptake by both the parent and mutant strains was inhibited by azide, an electron transport inhibitor, and also by *p*-chloromercuribenzoic acid, a mercurial sulfhydryl reagent. *N*-Ethylmaleimide, a sulfhydryl reagent, however, inhibited uptake by the mutant strain by only about 30% and did not inhibit uptake by the parent strain at all, even at 1.42 mM. Finally, 2,4-dinitrophenol,

a well-known uncoupler of oxidative phosphorylation, and arsenate were not very effective inhibitors of [^{3}H]adenosine uptake under the present experimental conditions.

Effect of osmotic shock on the ability to take up [^{14}C]showdomycin and [^{14}C]deoxycytidine

In order to test whether or not the specific components for the transport of the nucleosides studied are shock-releasable, the parent and mutant cells were subjected to cold-osmotic shock, as described by Nossal and Heppel[6].

TABLE III

THE REDUCTION AND RESTORATION OF [^{14}C]SHOWDOMYCIN, [^{14}C]DEOXYCYTIDINE, [^{14}C]URACIL, AND [^{14}C]LEUCINE UPTAKE BY OSMOTICALLY SHOCKED CELLS OF PARENT AND MUTANT STRAINS

The shock fluids and the shocked cells were obtained as described in Materials and Methods. 1 ml of the parent shock fluid was obtained from $5.1 \cdot 10^9$ parent cells and 1 ml of the mutant shock fluid from $5.7 \cdot 10^9$ mutant cells. The parent shocked cell suspension contained $6.7 \cdot 10^8$ cells per ml; the mutant shocked cell suspension, $6.9 \cdot 10^8$ cells per ml. Intact cells were also suspended in the same buffer solution at final concentrations of $7.0 \cdot 10^8$–$7.3 \cdot 10^8$ cells per ml. These cell suspensions were mixed with equal volumes of the shock fluids or with 0.5 mM $MgCl_2$ (aqueous solution), incubated at 37 °C for 6 min and stored in an ice-bath. After the mixtures had been preincubated for 10 min at 37 °C, 1-ml samples were added to equal volumes of minimal medium containing [^{14}C]showdomycin (321752 cpm/μmole, 18 μM final concentration), [^{14}C]uracil ($2162 \cdot 10^3$ cpm/μmole, 10 μM final concentration), [2-^{14}C]deoxycytidine ($216 \cdot 10^4$ cpm/μmole, 10 μM final concentration) or [U-^{14}C]leucine ($198 \cdot 10^4$ cpm/μmole, 10 μM final concentration). After a 4-min incubation, the cells were filtered, washed, and their total radioactivity determined. For further explanation, see text.

Cell	*Shock fluid*	*Uptake [10^{-18} mole/cell]*			
		[^{14}C]*Showdomycin* (%)	[^{14}C]*Deoxycytidine* (%)	[^{14}C]*Uracil* (%)	[^{14}C]*Leucine* (%)
Intact parent	—	1.44 (100)	2.00 (100)	1.62 (100)	0.86 (100)
Shocked parent	—	0.54 (38)	0.15 (8)	0.13 (8)	0.13 (15)
Shocked parent	Parent	0.83 (58)	1.57 (79)	1.35 (83)	0.51 (59)
Shocked parent	Mutant	0.76 (53)	1.73 (87)	1.23 (76)	0.49 (57)
—	Parent	0.000 *	0.001 *	0.009 *	0.006 *
Intact mutant	—	0.084 (100)	2.24 (100)	3.20 (100)	1.31 (100)
Shocked mutant	—	0.073 (87)	0.46 (21)	0.44 (14)	0.29 (22)
Shocked mutant	Mutant	0.068 (81)	1.39 (62)	2.18 (68)	0.79 (60)
Shocked mutant	Parent	0.056 (67)	1.42 (63)	2.27 (71)	0.86 (66)
—	Mutant	0.000 *	0.000 *	0.003 *	0.007 *

* 10^{-18} mole/0.5 ml shock fluid.

As indicated in Table III, whole cells of the parent and mutant subjected to the osmotic shock lost the ability to take up [^{14}C]deoxycytidine, [^{14}C]uracil, [^{14}C]-leucine, and [^{14}C]showdomycin. It is to be noted that the uptake, reduced due to the osmotic shock, could be partly restored if the shocked cells were incubated with shock fluid. There was no significant difference in the effectiveness of parent and mutant shock fluid for restoration of uptake of these materials. Neither the parent shock fluid nor the mutant shock fluid restored [^{14}C]showdomycin uptake by mutant shocked cells. Table IV shows the effect of deoxyguanosine on the shock-fluid-directed uptake of [^{14}C]deoxycytidine by parent and mutant shocked cells.

The parent-shock-fluid-directed uptake of [^{14}C]deoxycytidine by the mutant shocked cells was markedly inhibited by deoxyguanosine, as was the uptake of [^{14}C]deoxycytidine by intact mutant cells. The mutant-shock-fluid-directed uptake of [^{14}C]-deoxycytidine by parent shocked cells was not affected by deoxyguanosine, nor was the uptake of [^{14}C]deoxycytidine by intact parent cells. These results suggest that osmotic shock reduces the ability of the parent and mutant cells to take up deoxycytidine without changing their basic properties; the specific components of the deoxycytidine and showdomycin transport systems still appear to remain in the shocked cells.

TABLE IV

EFFECT OF DEOXYGUANOSINE ON SHOCK FLUID-DIRECTED [^{14}C]DEOXYCYTIDINE UPTAKE BY SHOCKED CELLS

The shock fluids and the shocked cells were obtained as described in Materials and Methods. 1 ml of the parent shock fluid was obtained from $3.9 \cdot 10^9$ cells and 1 ml of the mutant shock fluid from $3.7 \cdot 10^9$ cells. The parent shocked cell suspension contained $3.4 \cdot 10^8$ cells per ml; the mutant shocked cell suspension, $3.2 \cdot 10^8$ cells per ml; the parent intact cell suspension, $3.6 \cdot 10^8$ cells per ml; the mutant intact cell suspension, $3.3 \cdot 10^8$ cells per ml. These cell suspensions were mixed with equal volumes of the shock fluids or with 0.5 mM $MgCl_2$ (aqueous solution), incubated at 37 °C for 6 min and stored in an ice-bath. After the mixtures had been preincubated at 37 °C for 10 min, 1-ml samples were added to equal volumes of minimal medium containing [2-^{14}C]deoxycytidine ($2442 \cdot 10^3$ cpm/μmole, 10 μM final concentration), with or without deoxyguanosine (81 μM final concentration). After a 6-min incubation, the cells were filtered, washed, and their total radioactivity was determined. For further explanation, see text.

Cell	*Shock fluid*	[^{14}C]*Deoxycytidine uptake* *	
		Without deoxyguanosine	*With deoxyguanosine* **
Intact parent	—	2.46	2.73
Shocked parent	—	0.43	0.44
Shocked parent	Parent	1.52	1.39
Shocked parent	Mutant	1.61	1.49
Intact mutant	—	2.60	0.77
Shocked mutant	—	0.89	0.26
Shocked mutant	Mutant	1.92	0.66
Shocked mutant	Parent	1.79	0.54

* 10^{-18} mole/cell.
** 81 μM final concentration.

DISCUSSION

We showed in our previous papers[3,5] that *Escherichia coli* K-12 possesses at least two constitutive transport systems for each nucleoside. One system, or at least a part of the system, was common to a wide variety of nucleosides and also to the antibiotic showdomycin. The present report has presented evidence for further distinctions between these transport systems.

Deoxycytidine transport by *Escherichia coli* K-12 would seem to be mediated by two different transport systems; one is largely inhibited by adenine and pyrimidine nucleosides but only slightly inhibited by guanine and hypoxanthine nucleosides, the other is largely inhibited not only by adenine and pyrimidine nucleosides but

also by guanine and hypoxanthine nucleosides (it has not yet been examined whether deoxycytidine transport is inhibited by other nucleosides in a purely competitive manner). The former system was not observed in the showdomycin-resistant mutant (Shm^r-001), while the latter system (designated temporarily as the second deoxycytidine transport system) was retained. This fact, together with the previous observation that the showdomycin transport system is largely inhibited by adenine and pyrimidine nucleosides but only slightly inhibited by guanine and hypoxanthine nucleosides[5], strongly suggests that the former system is involved in showdomycin transport.

Deoxyguanosine transport by *Escherichia coli* K-12 would also seem to be mediated by two different transport systems; one is markedly inhibited by deoxycytidine, the other is not at all affected by the nucleoside. The former system was not detected in the showdomycin-resistant mutant (Shm^r-001), suggesting that at least a part of the system is common to showdomycin and deoxycytidine transport. The latter system (designated temporarily as the second deoxyguanosine transport system) was retained in the mutant strain.

Adenosine transport by *Escherichia coli* K-12 would seem to be mediated by two different transport systems; one is largely inhibited by deoxyadenosine, deoxycytidine, and deoxythymidine but only slightly inhibited by guanosine, the other system is only slightly inhibited by all these nucleosides (in the presence of caffeine). The former system possesses a high affinity for adenosine while the latter system possesses a low affinity for the nucleoside (in the presence of caffeine). The former system was not found in the showdomycin-resistant mutant (Shm^r-001), suggesting that it is the common transport system found for deoxycytidine and deoxyguanosine transport. The latter system (designated temporarily as the second adenosine transport) system was retained in the mutant strain.

The common transport system would be coupled with an electron transfer system and contain a specific component which is sensitive to *p*-chloromercuribenzoic acid but resistant to *N*-ethylmaleimide and to iodoacetic acid (in the presence of caffeine).

Peterson and coworkers[1,2] have reported that the apparent binding constants for cellular cytidine deamination and for cellular adenosine deamination are 10- and 25-fold lower than those for the cell-free preparations, respectively, and they have concluded that there is a transport pump(s) capable of saturating the internally located enzymes at external concentrations well below the K_m values for these enzymes. The mutant strain (Shm^r-001) lacking the common transport system for nucleosides showed a marked reduction in the ability to metabolize deoxycytidine and adenosine to uracil and inosine, respectively. This suggests that the common transport system actively concentrates deoxycytidine and adenosine at the site where cytidine deaminase and adenosine deaminase are located, whereas the second deoxycytidine transport system and the second adenosine transport system cannot transport low exogenous concentrations of deoxycytidine and adenosine into the cells sufficiently to saturate these respective enzymes.

Although the second deoxycytidine transport system was markedly inhibited by deoxyguanosine, the second deoxyguanosine transport system was not at all inhibited by deoxycytidine. This suggests that these systems are distinct from one another and that deoxyguanosine inhibits the second deoxycytidine transport

system without being transported itself by the second deoxycytidine transport system.

Although it remains to be elucidated whether or not the second adenosine transport system is identical either with the second deoxycytidine transport system or with the second deoxyguanosine transport system, it may be important to note that adenosine transport by the showdomycin-resistant mutant (Shm^r-001) was only slightly inhibited by deoxycytidine even under incubation at 0.5 °C in the absence of caffeine (data not shown) and that the deoxyguanosine transport by the showdomycin-resistant mutant (Shm^r-001) was significantly inhibited by adenosine.

The second adenosine transport system is sensitive to an electron transport inhibitor and to a mercurial sulfhydryl reagent, as is the common transport system; but it contains an iodoacetic acid-sensitive component which is not present in the common transport system.

A variety of binding proteins implicated in the transport of amino acids, sugars, and phosphate have been isolated from *Escherichia coli*[9-17] by the cold-osmotic shock procedure of Neu and Heppel[8]. Our results indicate that the components responsible for specificity with respect to the two different deoxycytidine transport systems are not releasable from the cells by osmotic shock, although the uptake of deoxycytidine by shocked cells was enhanced by the addition of the shock fluid. It will be important to determine whether or not the membrane vesicles prepared by the method of Kaback[18] are capable of taking up deoxycytidine, showdomycin, and other nucleosides.

Peterson and Koch[1] have suggested that there are sites in the *Escherichia coli* cell membrane which carry adenosine permeases and can also react with pyrimidine nucleosides through a second permease, and that there are other transport sites of broader specificity in the membrane of the cell that can react with various nucleosides. According to their concept, it is possible to consider that the functional alterations observed in the showdomycin-resistant mutant (Shm^r-001) are located at the former sites in the bacterial membrane, while the latter sites remain intact, since the observed properties of the common transport system were similar to those described for the system containing the former sites.

Recent results revealed that the showdomycin-resistant mutant (Shm^r-001) shows strong enhancement of the rate of uptake of some amino acids (leucine, isoleucine, valine, proline and methionine). This will be described in subsequent reports.

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